CRE-LOX MEDIATED CONDITIONAL REMOVAL OF MYELOID CELL-DERIVED *Mif* IN A MOUSE MODEL OF ORAL CANCER.

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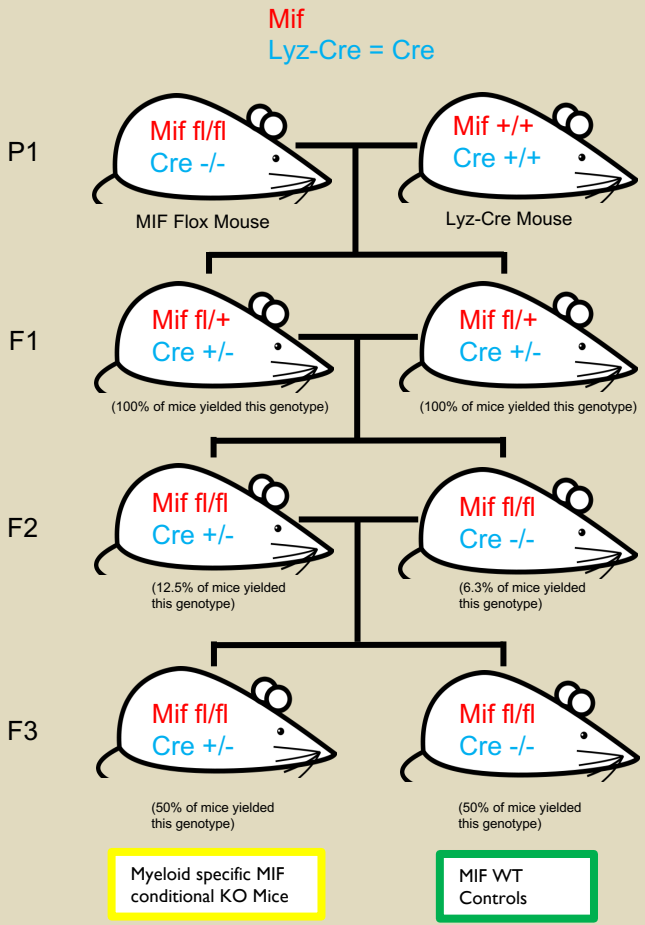
Oral cancer affects people globally with an estimated 657,000 new cases and 330,000 deaths yearly. Macrophage migration inhibitory factor (*Mif*) is a gene that encodes for a cytokine involved in regulating cell-mediated immunity, immunoregulation, and inflammation. Previous research has demonstrated that global *Mif* inhibition results in reduced oral cancer development. This was associated with a decrease in the accumulation of myeloid cell populations in the oral tumor microenvironment. Therefore, our group is interested in elucidating the role of *Mif* expression by myeloid populations in the oral tumor microenvironment. We previously generated floxed *Mif* reporter mice (*Mif* $^{Fl/Fl}$). Selective breeding between *Mif* $^{Fl/Fl}$ mice and *Cre* knock-in mice driven by the Lysozyme promoter (*Lyz-Cre*) was performed to produce progeny with *Mif* deficiency in myeloid cells. Genotypic characterization for *Mif* $^{Fl/Fl}$ *and Lyz-Cre* was performed by PCR of genomic DNA extracted from mouse ear tissue. Phenotypic characterization of myeloid *Mif* deficiency was performed through flow cytometric analysis on various myeloid cell populations. Selective breeding of the *Mif* $^{Fl/Fl}$ mice with the *Lyz-Cre* mice for three generations yielded myeloid-specific *Mif* deficient mice. Genotypic PCR characterization of Mif $^{Fl/Fl}$ mice yielded band sizes of 404 bp while wild-type mice yielded a band size of 221 bp. Furthermore, genotypic characterization of *Lyz-Cre* mice yielded a band size of 700 bp while wild-type mice yielded a band size of 350 bp. Phenotypic flow cytometric analysis of bone marrow-derived macrophages and peritoneal macrophages from myeloid-specific *Mif* deficient mice revealed absence of *Mif* expression in these cells. Furthermore, in these mice, this difference was not observed in lymphoid cells. Our data indicate that, using the Cre-LoxP system and selective breeding conditional *Mif* gene removal in myeloid cells in mice can be achieved to help us gain a better understanding of the role *Mif* plays in the context of oral cancer and other inflammatory diseases.

BACKGROUND

- Oral Cancer, 657,000 new cases and 330,000 deaths yearly[1]
- Reduction of inflammation for those with cancer is beneficial
- Macrophage migration inhibitory factor (*Mif*)
  - Regulates cell-mediated immunity, immunoregulation, and inflammation
  - Systemic inhibition leads to better prevention, prognoses and a decrease in tumor promoting myeloid-derived cell populations[2]
- The Cre recombinase and loxP system
  - LoxP sequence is inserted to flank the *Mif* gene, aka floxxed
  - Cre recombinase dimerizes with loxP and removes targeted *Mif* gene

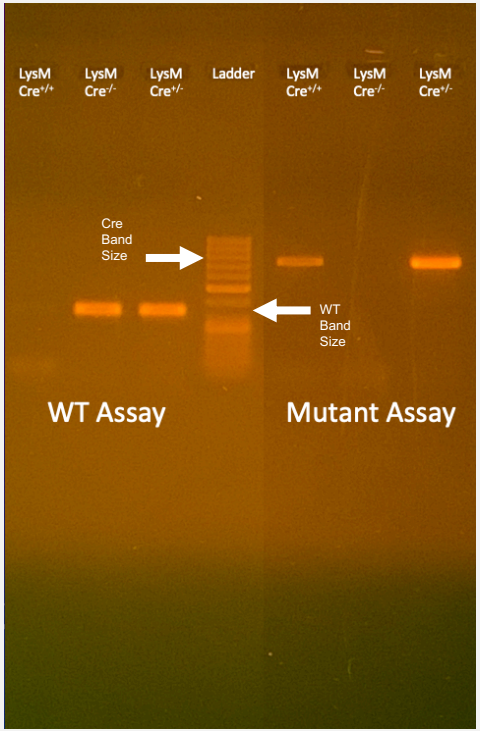


METHODS

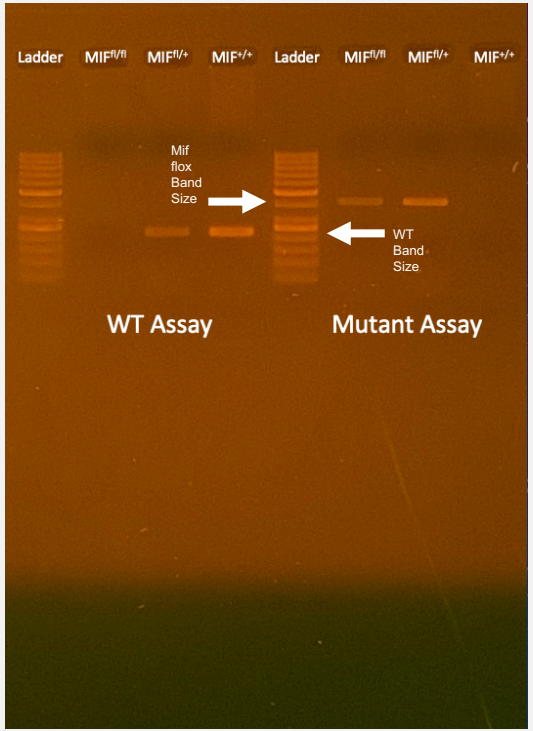
- Selective breeding strategy
- Genotypic characterization
  - Ear samples
- Phenotypic characterization
  - Flow Cytometric Analysis
    - Myeloid derived cells
      - Bone Marrow Derived Macrophages (BMDMs)
      - Bone Marrow Derived Dendritic Cells (BMDCs)
      - Peritoneal Macrophages
    - Lymphocytes
      - Splenocytes
      - Lymph Node derived cells

RESULTS – GENOTYPIC CHARACTERIZATION

Lyz-Cre Genotyping



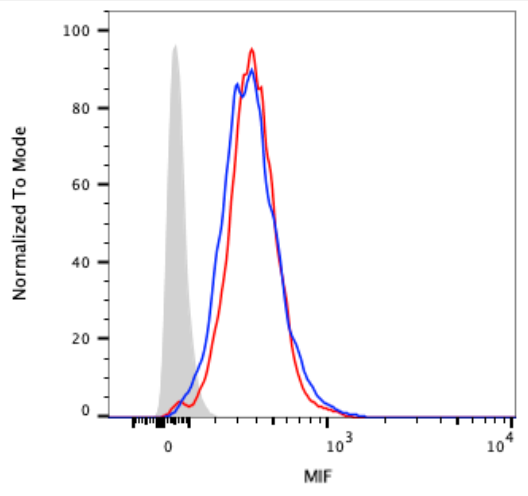
Mif Genotyping



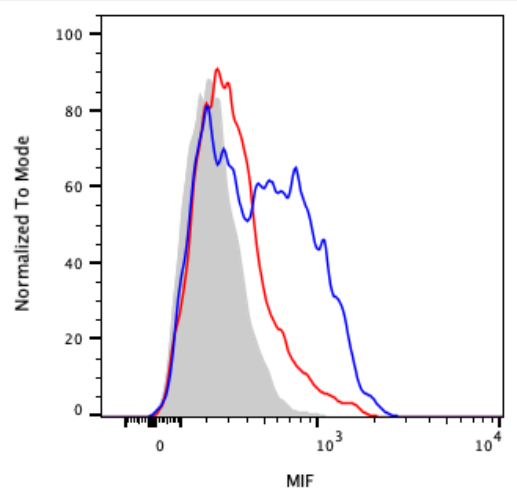
- Examples of each genotype
- Cre expected bands
  - WT 350 bp
  - Lyz-Cre Mutant 700 bp
- Mif Expected bands
  - WT Mif 221 bp
  - Mif fl 404 bp

RESULTS – PHENOTYPIC CHARACTERIZATION

CD4+ Cells



BMDMs



$MIF^{+/+}$ WT Control

$MIF^{fl/fl}$ Lyz-$Cre^{+/+}$ Myeloid KO

MIF Global KO Control

$MIF^{+/+}$ WT Control

$MIF^{fl/fl}$ Lyz-$Cre^{+/+}$ Myeloid KO

MIF Global KO Control

- CD4 cells showed no difference in *Mif* from WT
- Myeloid-derived BMDMs did have a decrease in *Mif*

CONCLUSIONS

- Deletion of *Mif* in myeloid-derived cells through Cre-LoxP
  - Genotypic characterization
  - Phenotypic characterization
- Further analyses

Key References:

[1] "Oral Cancer." World Health Organization, World Health Organization, 12 Sept. 2018, www.who.int/cancer/prevention/diagnosis-screening/oral-cancer/en/.

[2] Oghumu, Steve, et al. "Deletion of Macrophage Migration Inhibitory Factor Inhibits Murine Oral Carcinogenesis: Potential Role for Chronic pro-Inflammatory Immune Mediators." International Journal of Cancer, U.S. National Library of Medicine, 15 Sept. 2016, www.ncbi.nlm.nih.gov/pubmed/27164411.

THANK YOU!